

# SEQUENCE LISTINGS

<110> Hanmi Pharm. Co., Ltd.

<120> EXPRESSION VECTOR FOR SECRETING ANTIBODY FRAGMENT USING E. COLI  
SIGNAL SEQUENCE AND METHOD FOR MASS-PRODUCING ANTIBODY FRAGMENT

<130> Q94300

<140> 10/576,068

<141> 2006-04-14

<150> KR1020030072216

<151> 2003-10-16

<150> PCT/KR04/02625

<151> 2004-10-14

<160> 36

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<220>

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<223> gene fragment of light chain variable region

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<210> 4  
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<400> 4  
aggctgtagg ctgctgatgg tgagagtga atctgtccca gatccactgc cactgaaccg 60  
agatgggacc cctgattgca 80

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<220>  
<223> gene fragment of light chain variable region

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ccatcagcag cctacagcct gaagatgttg caacttatta ctgtcaaagg tataaccgtg 60  
cacccgtatac ttttggccag 80

<210> 6  
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<210> 7  
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<212> DNA  
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<400> 7

gggaagcttc gatcggaggt gcagctggtg gagtctgggg gaggcttggt acagcccggc 60  
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<210> 8  
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<400> 8  
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 gagtctcagg gacctgccg 79

<210> 9  
 <211> 80  
 <212> DNA  
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<220>  
 <223> gene fragment of heavy chain variable region

<400> 9  
 tgcactgggt ccggcaagct ccaggggaagg gcttgggaatg ggtctcagct atcacttgga 60  
 atagtgggtca catagactat 80

<210> 10  
 <211> 80  
 <212> DNA  
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<220>  
 <223> gene fragment of heavy chain variable region

<400> 10  
 atacagggag ttcttggcgt tgtctctgga gatgggtgaat cggccctcca cagagtccgc 60  
 atagtctatg tgaccactat 80

<210> 11  
 <211> 80  
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 <223> gene fragment of heavy chain variable region

<400> 11

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attactgtgc gaaagtctcg 80

<210> 12  
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<400> 12  
cactcgagac ggtgaccagg gtaccttggc cccaatagtc aaggaggagac gcggtgctaa 60  
ggtacgagac tttcgcacag taat 84

<210> 13  
<211> 39  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> RT-PCR forward primer specific for heavy chain

<400> 13  
cccaagctta ggctccacc aagggcccat cgggtcttcc 39

<210> 14  
<211> 48  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> RT-PCR reverse primer specific for heavy chain

<400> 14  
gggggatcct tatgggcacg gtgggcatgt gtgagttttg tcacaaga 48

<210> 15  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> RT-PCR forward primer specific for light chain

<400> 15  
cccaagcttt cgcgaactgt ggctgcacca tctgtcttca tc 42

<210> 16

<211> 42  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> RT-PCR reverse primer specific for light chain  
  
 <400> 16  
 cccggatccc taacactctc ccctgttgaa gctctttgtg ac 42  
  
 <210> 17  
 <211> 69  
 <212> DNA  
 <213> modified E. coli thermostable enterotoxin II signal sequence  
  
 <400> 17  
 atgaaaaaga caatcgcat tcttcttgca tctatgttcg ttttttctat tgctacaaat 60  
 gcccgaggcg 69  
  
 <210> 18  
 <211> 45  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> forward primer containing StuI restriction enzyme site  
  
 <400> 18  
 tctattgcta caaatgccca ggccttccca accattccct tatcc 45  
  
 <210> 19  
 <211> 45  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> reverse primer containing StuI restriction enzyme site  
  
 <400> 19  
 agataacgat gtttacgggt ccggaagggt tggttaaggga atagg 45  
  
 <210> 20  
 <211> 51  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> reverse primer specific for light chain  
  
 <400> 20  
 gggggatcct cacgcggcgc atgtgtgagt tttgtcacia gatttaggct c 51

<210> 21  
 <211> 43  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> forward primer containing SD sequence and BamHI restriction enzyme site  
  
 <400> 21  
 gggggatcca ggaggtgatt tatgaaaaag acaatcgcat ttc 43  
  
 <210> 22  
 <211> 44  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> forward primer containing BpuI restriction enzyme site  
  
 <400> 22  
 ggggctgagc aggaggtgat ttatgaaaaa gacaatcgca tttc 44  
  
 <210> 23  
 <211> 52  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> reverse primer containing BpuI restriction enzyme site  
  
 <400> 23  
 ggggctcagc tcacgcggcg catgtgtgag ttttgtcaca agatttaggc tc 52  
  
 <210> 24  
 <211> 63  
 <212> DNA  
 <213> E. coli OmpA signal sequence  
  
 <400> 24  
 atgaaaaaga cagctatcgc gattgcagtg gcactggctg gtttcgctac cgttgcgcaa 60  
 gct 63  
  
 <210> 25  
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 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> forward primer specific for heavy chain

<400> 25  
gaggttcagc tagtcgagtc aggaggcggc 30

<210> 26  
<211> 51  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing HindIII and StuI restriction enzyme sites

<400> 26  
gggagatcctt cacgcggcgc atgtgtgagt ttgtcacaa gatttaggct c 51

<210> 27  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 27  
gacattcaaa tgacccagag cccatccagc 30

<210> 28  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing HindIII and NruI restriction enzyme sites

<400> 28  
cccagatctc taacactctc ccctgttgaa gctctttgtg ac 42

<210> 29  
<211> 41  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 29  
ggggtcgaca ggagggtgatt tatgaaaaag acagctatcg c 41

<210> 30  
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 <212> DNA  
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 <210> 31  
 <211> 42  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> forward primer specific for modified E. coli enterotoxin II  
 signal peptide and containing NdeI restriction enzyme site  
  
  
 <400> 31  
 gggcatatga aaaagacaat cgcatttctt cttgcatcta tg 42  
  
  
 <210> 32  
 <211> 705  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> TNF-alpha heavy chain  
  
  
 <400> 32  
 gaggttcagc tagtcgagtc aggaggcggg ttggtacagc ccggcaggtc cctgagactc 60  
 tcctgtgcgg cctctggatt cacctttgat gattatgcc a tgactgggt ccggcaagct 120  
 ccaggggaagg gcttggaaatg ggtctcagct atcacttgga atagtggta catagactat 180  
 gcggactctg tggagggccg attcaccatc tccagagaca acgccaagaa ctccctgtat 240  
 ctgcaaatga acagtctgag agctgaggat acggccgtat attactgtgc gaaagtctcg 300  
 taccttagca ccgcgtcctc ccttgactat tggggccaag gtaccctggg caccgtctcg 360  
 agtgccctca ccaagggccc atcgggtctc cccctggcac cctcctccaa gagcacctct 420  
 gggggcacag cggccctggg ctgcctgggc aaggactact tccccgaacc ggtgacggtg 480  
 tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggctgt cctacagtec 540  
 tcaggactct actccctcag cagcgtgggt accgtgccct ccagcagctt gggcacccag 600  
 acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag 660



cccaaattctt gtgacaaaac tcacacatgc ccaccgtgcc catag 705

<210> 33  
<211> 645  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> TNF-alpha light chain

<400> 33  
gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtagggga cagagtcacc 60  
atcacttgtc gggcaagtca gggcatcaga aattacttag cctggtatca gcaaaaacca 120  
gggaaagccc ctaagctcct gatctatgct gcatccactt tgcaatcagg ggtcccatct 180  
cggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag cctacagcct 240  
gaagatgttg caacttatta ctgtcaaagg tataaccgtg caccgtatac ttttggccag 300  
gggaccaagg tggaaatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcc 360  
tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 420  
cccagagagg ccaaagtaca gtggaagggtg gataacgccc tccaatcggg taactcccag 480  
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540  
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600  
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag 645

<210> 34  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Recombinant light chain of TNF-alpha Fab'

<400> 34  
Asp Ile Gln Met Thr Gln Ser  
1 5

<210> 35  
<211> 8  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Recombinant heavy chain of TNF-alpha Fab'

<400> 35  
Glu Val Gln Leu Glu Val Asp Ser  
1 5